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14. ABSTRACT The goal of this project is to evaluate the hypothesis that traumatic brain injury induces alterations in the brain's reward circuitry which may make an injured brain more susceptible to the rewarding effects of opioids. We are currently conducting experiments to evaluate the hypothesis that TBI causes changes in the analgesic response to opioids following acute and repeated drug administration. We are secondly in the midst of testing the hypothesis that moderate TBI increases the susceptibility for opioid abuse as measured by an alteration in the rewarding properties of oxycodone. We have completed the first year of experimentation and thus far have found that the mean duration of transient unconsciousness in the animals that received TBI is consistent with a moderate injury. A trend toward increase tail withdrawal latencies was observed in the TBI group, but the number of animals per group is yet not sufficient for complete analysis. We have also observed a trend for differences between potency of oxycodone administration between TBI and sham rats. Trends for between groups differences were also seen in self-administration experiments. All studies are on-going.					
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Introduction:

Progress Report for DoD Peer Reviewed Medical Research Program of the Office of the Congressionally Directed Medical Research Program FY10 Investigator-Initiated Research Award: Partnering PI Option Application entitled “Opioid Abuse after TBI”

This report was prepared by Candace L. Floyd, Ph.D. for the work conducted in collaboration with Katherine L. Nicholson, DVM, Ph.D.

Our progress in completion of the aims is detailed below. This report focuses on the work conducted at UAB. However, it is important to emphasize that Dr. Floyd and her staff traveled to VCU to induce the traumatic brain injury (TBI) in all animals, thus the tables of work complete are listed as well. A brief description of the aims, the work conducted, and the data collected respective to the statement of work is provided after the original text of the aims and statement of work, which are denoted by bolded and italicized font, respectively.

Aim 1: Evaluate the hypothesis that moderate TBI causes changes in the analgesic response to opioids following acute and repeated drug administration.

Aim 2: Investigate the hypothesis that moderate TBI increases the susceptibility for opioid abuse as measured by an alteration in the rewarding properties of oxycodone.

Aim 3: Evaluate the propensity for development of physical dependence to opioids following moderate TBI.

Body:

Progress on year 2 tasks to be conducted exclusively at UAB:

YEAR 2:

- *Task 1: Travel to VCU to induce lateral fluid percussion TBI in rats in months 3, 5, and 9, 10, 11 and 12 of year 2, as described above*

Completed as described above.

- *Task 2: Continue histological and biochemical analysis of cell death/ gliosis, DA signaling, opioid receptor number and growth factors from rodent brains received from VCU*

This task is on-going. The procedures which are currently being carried out are summarized below. Preliminary data to date follows.

a. Tissue preparation

Animals were deeply anaesthetized and perfused. Brains were harvested and serial random sections were sectioned on a cryostat and collected from Bregma -0.8mm to -4.8mm, encompassing the cortical region at the injury epicenter as well as the entire hippocampal formation.

b. Cresyl violet histochemistry

Cresyl violet histological processing of tissue stains Nissl substance, which is composed mostly of rough endoplasmic reticulum and is lost after neuronal injury or axonal degeneration. See examples of histology in figures 1 and 2. Preliminary results show that traumatic brain injury induced a loss of neurons in the dentate gyrus and that this loss was relatively consistent across oxycodone self-administration groups (see figure 3).

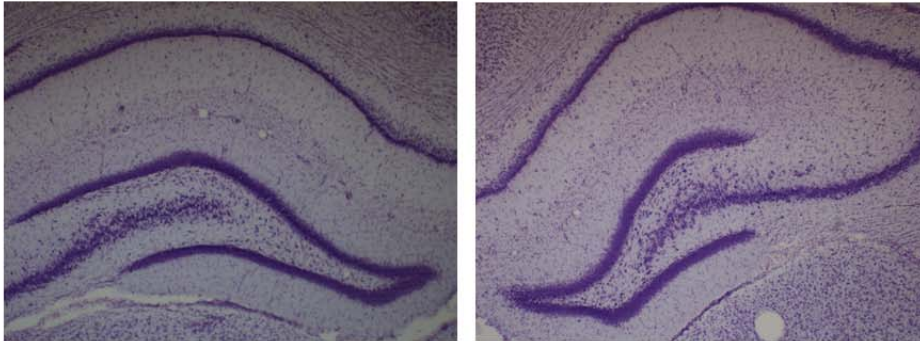


Figure 1: Representative micrographs of contralateral (left panel) and ipsilateral (right panel) dentate gyri from a sham animal that underwent oxytocin self-administration.

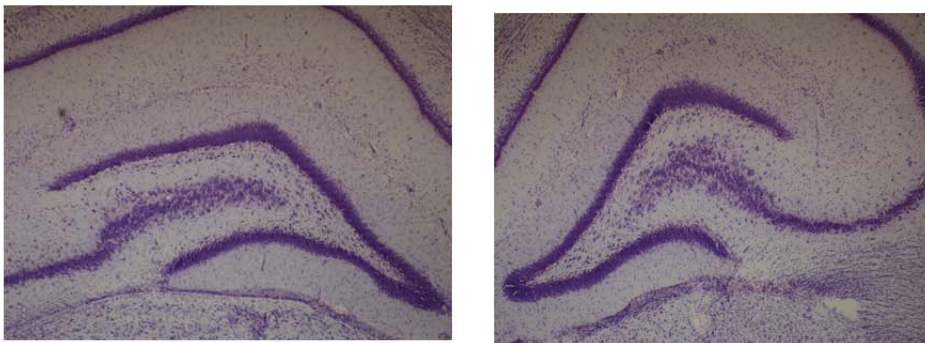


Figure 2: Representative micrographs of contralateral (left panel) and ipsilateral (right panel) dentate gyri from a TBI animal that underwent oxytocin self-administration

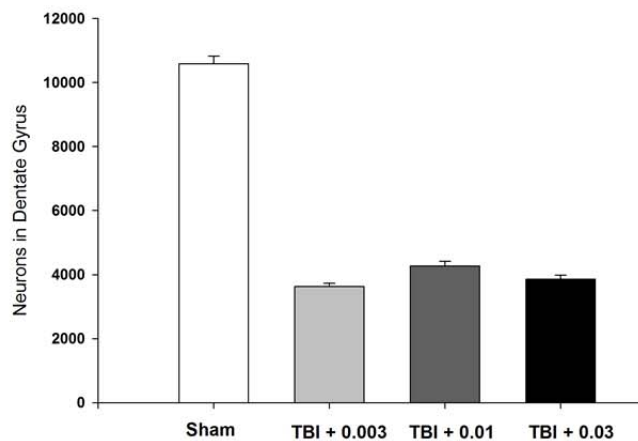


Figure 3: Evaluation of cell death in the dentate gyrus of oxycodone self-administration animals. Preliminary stereological evaluation of numbers of neurons in the dentate gyrus in animals that conducted behavioral analysis in Virginia. No statistical evaluation was conducted as the analysis is incomplete.

c. Other on-going histological assessments include immunohistochemistry for:

- Reactive glial response is being determined by measuring the luminance intensity of GFAP staining
- Necrotic and apoptotic cell death by immunohistochemistry for Fluoro-Jade B and Caspase-3
- -- CD11b is a cell marker found on the plasma membrane of activated microglia and is being used as an indicator of inflammation.

d. Immunoblotting for quantification of Mu Opioid Receptor and Dopamine receptors

Immunoblotting was conducted using standard techniques as we have previously described. We are currently examining the expression of the mu opioid receptor at 5 days post-injury in the following brain regions: amygdala, nucleus accumbens, frontal cortex, and ventral tegmental area (VTA). Representative immunoblot for the mu opioid receptor expression in amygdala and VTA from sham and TBI animals indicated an increased expression in both regions after TBI (figure 4). This increase was confirmed upon quantification with densitometry (figure 5) in that elevated levels of mu opioid receptor expression were quantified in the amygdala, nucleus accumbens, frontal cortex and VTA.

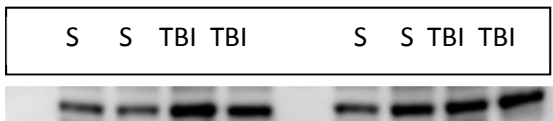


Figure 4: Representative immunoblot for mu opioid receptor from amygdala and VTA at 5 days post-TBI. Lanes (left to right): amygdala sham, sham, TBI, TBI; VTA sham, sham TBI, TBI.

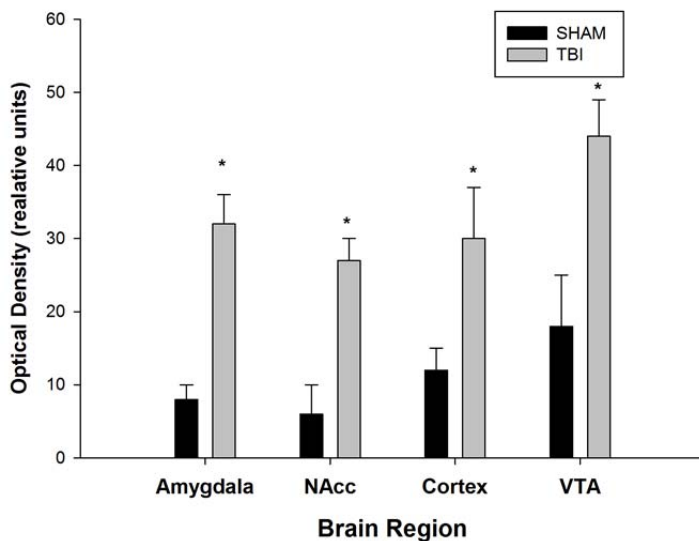


Figure 5: Quantification of expression of mu opioid receptor expression at 5 days post-TBI. The brain regions examined were amygdala, nucleus accumbens (NAcc), frontal cortex, and ventral tegmental area (VTA). Black bars are from sham animals and gray are from animals that received TBI. *=p<.05

Similarly, we are currently evaluating the expression of the dopamine receptor 1 (D1) in the same brain regions at 5 days post-TBI and a representative immunoblot is shown in figure 6. Upon preliminary quantification with densitometry (figure 7), it appears that expression of the D1 receptor is down-regulated after TBI. Although the numbers of animals is below that indicated in a power analysis for determination of statistical significance, if these trends continue it would seem that TBI induces an acute reduction in D1 expression throughout the reward circuitry.

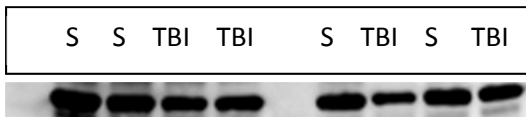


Figure 6: Representative immunoblot for dopamine receptor 1 from amygdala and VTA at 5 days post-TBI. Lanes (left to right): amygdala sham, sham, TBI, TBI; VTA sham, TBI, sham, TBI

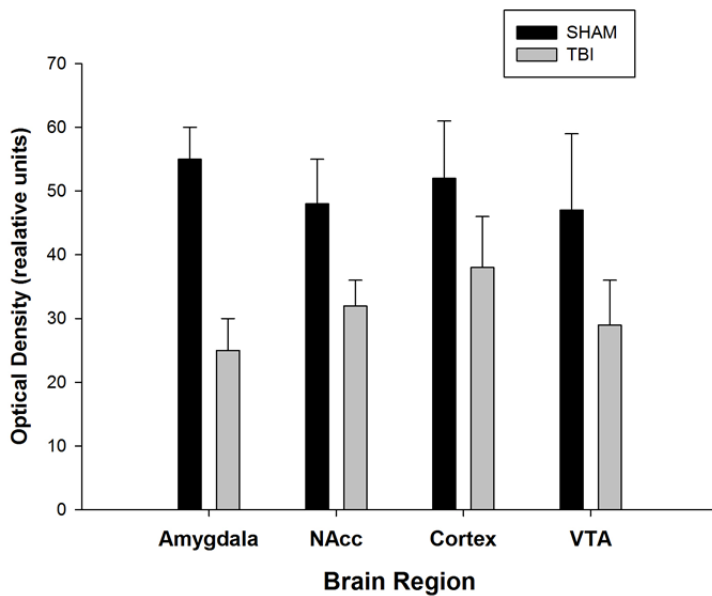


Figure 7: Quantification of expression of dopamine receptor 1 expression at 5 days post-TBI. The brain regions examined were amygdala, nucleus accumbens (NAcc), frontal cortex, and ventral tegmental area (VTA). Black bars are from sham animals and gray are from animals that received TBI. Statistical analysis was not conducted as experiment is on-going and below power analysis for detection of statistical significance.

In parallel, , we are assessing the expression of the dopamine receptor 2 (D2) in the same brain regions at 5 days post-TBI and a representative immunoblot is shown in figure 8. Upon preliminary quantification with densitometry (figure 9) , it appears that expression of the D2 receptor is not substantially altered after TBI. Although the numbers of animals is below that indicated in a power analysis for determination of statistical significance, if these trends continue it would seem that TBI does not significantly affect the expression of D2 receptors in the brain regions examined.

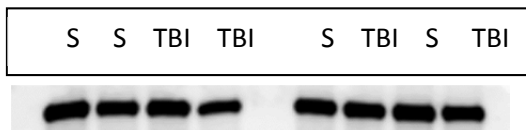


Figure 8: Representative immunoblot for dopamine receptor 2 from amygdala and VTA at 5 days post-TBI. Lanes (left to right): amygdala sham, sham, TBI , TBI; VTA sham, TBI, sham, TBI.

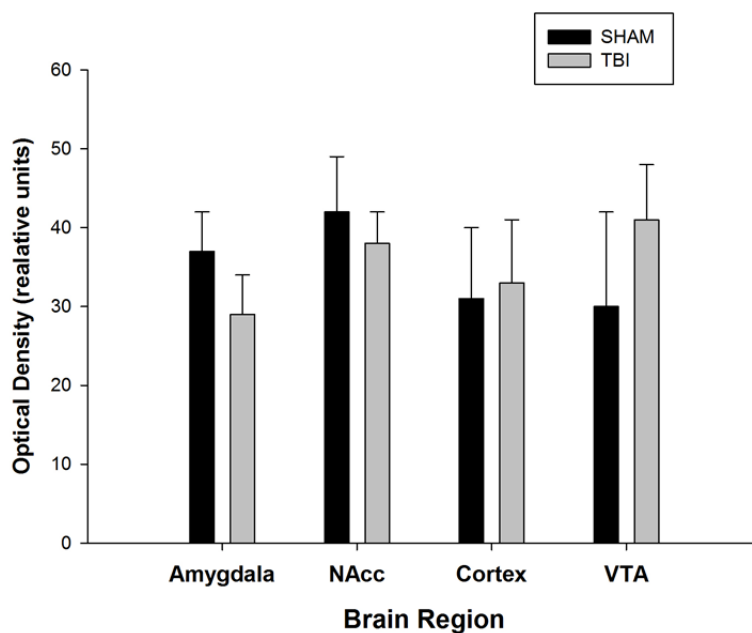


Figure 9: Quantification of expression of dopamine subtype 2 receptor expression at 5 days post-TBI. The brain regions examined were amygdala, nucleus accumbens (NAcc), frontal cortex, and ventral tegmental area (VTA). Black bars are from sham animals and gray are from animals that received TBI.

- *Task 3: Lead preparation of abstracts / posters to report scientific discoveries obtained from analysis of TBI-induced alteration in reward circuitry, opioid neurotransmission and neurotrophic factors*

We assisted with submission of an abstract to the Society for Neuroscience Meeting in 2012. Additionally, we recently presented data at the 2013 National Neurotrauma Society meeting and that poster/abstract is below. We anticipate a manuscript to be completed by December 2013, with more to follow.

Key Research Accomplishments:

- Preliminary data indicate hippocampal cell death in all TBI groups, regardless of oxycodone self-administration.
- Preliminary data indicate changes in expression of mu opioid receptor and dopamine subtype 1 receptor after TBI.

Reportable Outcomes:

- Presentation of data at 2013 National Neurotrauma Society Annual Meeting

Conclusion:

The data thus far suggest that moderate/severe traumatic brain injury induces a change in the response to oxycodone such that injured subjects are more likely to abuse oxycodone and less sensitive to the negative effects the drug. This is likely due to changes in the brain reward circuitry induced by injury including increases in mu opioid receptor expression and decreases in D1 receptor expression.

Reference List

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- (2) Schmued LC, Hopkins KJ. Fluoro-Jade B: a high affinity fluorescent marker for the localization of neuronal degeneration. *Brain Res* 2000;874(2):123-130.
- (3) Day NL, Floyd CL, D'Alessandro TL, Hubbard WJ, Chaudry IH. 17beta-estradiol confers protection following traumatic brain injury in the rat and involves activation of G Protein-coupled estrogen receptor 1 (GPER). *J Neurotrauma* 2013.

Effects of Oxycodone Following Traumatic Brain Injury in Rats

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INTRODUCTION:

Epidemiological data indicate that drug abuse rates increase following traumatic brain injury (TBI), but the underlying reasons remain unclear^{1,2}. One of the most commonly prescribed and also misused/abused prescription pain medications is oxycodone, especially in its sustained release formulation OxyContin³. This pain medication and others in the same class of drugs, opioids, can produce euphoric effects. Additionally, long term administration of opioid compounds can produce alterations in brain chemistry that exacerbate the risk of addiction³. There is significant overlap in anatomical brain regions involved in reward pathways associated with addiction and the brain regions commonly damaged in TBI.

Thus, the goal of this research was to evaluate the effect of oxycodone administration after TBI in a rat model.

METHODS:

Experimental Groups:

- Adult male rats (2 months old) were used.
- Moderate-severe lateral fluid percussion injury (injured) or craniectomy only (sham)

Oxycodone Self-Administration:

- 5 days prior to injury, all animals received surgical implantation of a chronic indwelling venous catheter
- Beginning at 5 days after TBI or sham procedure, animals were tested in the self-administration acquisition procedure in standard operant chambers in daily sessions
- During the session, a single response, fixed ratio 1 resulted in the delivery of 0.1ml, 3 sec infusion of one of either 0.003, 0.01, or 0.03 mg/kg oxycodone
- Following acquisition, the rats continued self-administering the acquisition dose until performance was stable (3 days >20% change)

Assessment of nociception with warm water tail withdrawal:

- Rats were habituated to the test apparatus (restraint tube with tail freely hanging out) and acclimated to tail immersion in 40 °C water
- 5 days prior to TBI, all rats were implanted with programmable minipumps (iPRECIO system) for oxycodone administration
- To mimic clinical exposure, oxycodone solution (or saline) was released every 6 hours beginning on day 5 post-TBI
- On test days, tails were submerged for 12 seconds in warm water at either 50°C or 55 °C, and the latency to withdrawal the tail was recorded

RESULTS:

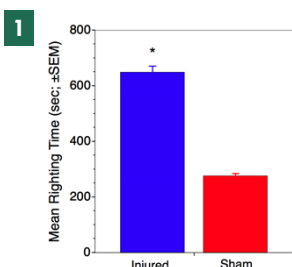


Figure 1: Duration of Transient Unconsciousness.

Injured animals exhibited an increased suppression of righting reflex as compared to sham. * $p < .05$.

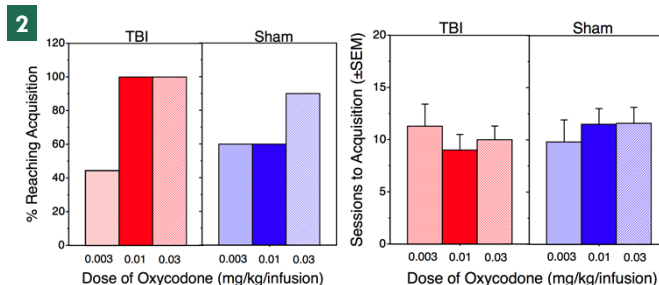


Figure 2: Effect of injury on oxycodone self-administration.

A) TBI results in great percentage of rats acquiring oxycodone self-administration at the intermediate dose. B) TBI does not affect the rate of acquisition.

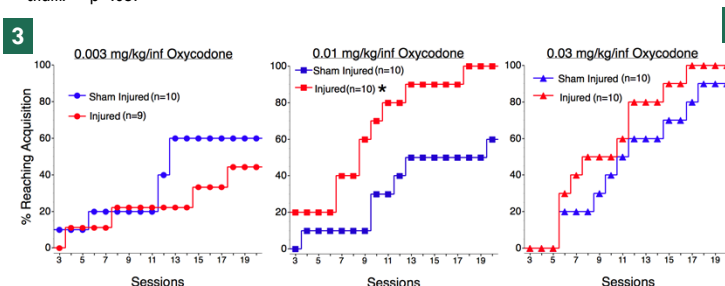


Figure 3: Acquisition at the intermediate dose of oxycodone was greater in the TBI group. Left to right panels compare the percent of the group that reached the acquisition criteria of 3 days responding at > 15 infusions/session.

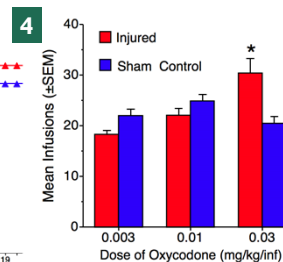


Figure 4: Infusion levels

The highest dose of oxycodone maintained significantly higher infusion levels in TBI group as compared to sham. * $p < 0.05$.

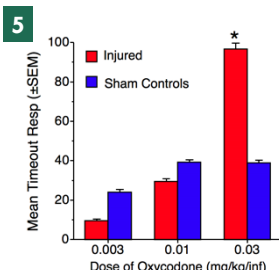


Figure 5: Time out responding

Animals in the TBI group responded significantly more during time outs at the highest dose of oxycodone. * $p < 0.05$

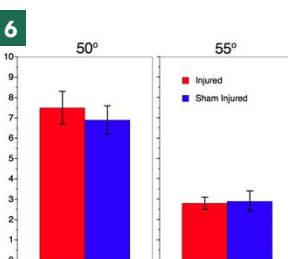


Figure 6: Nociceptive responses

Baseline nociceptive responses were not different between animals in the TBI or sham groups.

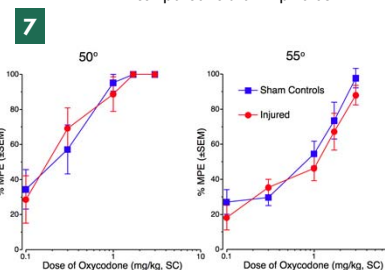
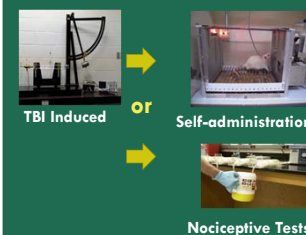


Figure 7: Anti-nociceptive responses

Acute anti-nociceptive effects of oxycodone were not different between animals in the TBI or sham groups.

SUMMARY OF METHODS:



CONCLUSIONS:

- TBI group showed an increased sensitivity to oxycodone's reinforcing effects as compared to sham group
- TBI group appeared to be less sensitive to oxycodone's "use-limiting" effects compared to the sham injured subjects
- TBI group exhibited higher timeout responding suggestive of increased impulsivity
- No differences were detected between groups in baseline nociception or in response to the acute anti-nociceptive effects of oxycodone

Taken together, these data indicate that TBI may enhance self-administration of oxycodone, particularly at the moderate dose.

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